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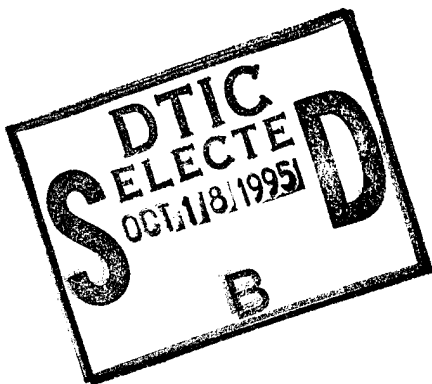
ANTIBODY-BASED FIBER OPTIC EVANESCENT WAVE SENSOR

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13. ABSTRACT (Maximum 200 words) An antibody-based fiber optic biosensor is described. This biosensor will serve as a model for future development of antibody-based detection of biological toxins. It is sensitive, specific, and new assays will be based solely on the antibody recognition element of the sensor.				
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EXECUTIVE SUMMARY

The fiber optic evanescent wave sensor uses the evanescent wave to excite fluorophores just outside the waveguide boundary. The resulting fluorescence is trapped and transmitted back up the fiber. To test proof of principle for an antibody based sensor, purified Rabbit immunoglobulin G (IgG) was immobilized on the surface of quartz fibers by adsorption or covalent binding and probed with fluorescein isothiocyanate-labeled Goat anti-Rabbit antibody (FITC-aR). The IgG density was quantitated by ^{125}I -Protein A binding. For covalent binding, fibers were silanized and activated with glutaraldehyde. There was no significant difference in initial rates and total binding between regular (non-silanized) and silanized fibers. Specific binding of FITC-aR was inhibited by non-labeled Goat anti-Rabbit (aR). This system is being used as a model for the development of antibody-based toxin detection assays.

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PREFACE

The work described in this report was authorized under Project No. 10162622A553. This work was started in October 1990 and completed in September 1991.

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ANTIBODY-BASED FIBER OPTIC EVANESCENT WAVE SENSOR

1. INTRODUCTION

Biological molecules bind very selectively to specific target sites within their native environments. Biosensors take advantage of this bioaffinity by immobilizing the target sites on various transductive devices and converting biomolecular interactions on the surface into electrical or optical signals. Capacitance, fiber optic, ChemFET, and potentiometric devices all have been designed and tested using receptors and antibodies as the sensor coating.¹⁻⁴

The fiber optic evanescent wave sensor detects molecules within the evanescent wave zone which extends only a fraction of a wavelength above the surface of the fiber. Binding events on the surface of the fiber are easily monitored without interference from the bulk solution. Excitation of fluorophores occurs when some of the light transmitted down the fiber enters the evanescent zone. Fluorophore emission at a higher wavelength is trapped in the fiber and is transmitted back through the fiber to a detector.

In the present study, we developed a model antibody system using Rabbit immunoglobulin G (IgG) immobilized on the surface of quartz rods. IgG was either covalently immobilized on silanized fibers, or passively adsorbed on non-silanized fibers. When fluorescein isothiocyanate-labeled anti-Rabbit (FITC-aR) was perfused across the IgG immobilized fiber, FITC-aR bound to the IgG producing a fluorescent signal that was directly proportional to the amount of antigen bound. Perfusion of fibers with increasing amounts of unlabeled aR also decreased the signal, due to competition for the same binding sites.

Protein A, isolated from Staphylococcus aureus, was used to quantitate the amount of protein on the fiber. Since Protein A binds to the Fc region of the antibody without interfering with the antigen binding, it may be used to probe a number of immunoglobulin molecules. The simplicity of this model system is that, by changing antibodies on the fiber, any molecule for which antibodies are available may be detected.

2. MATERIALS AND METHODS

2.1 Biochemicals Used.

Rabbit immunoglobulin G (IgG) was obtained from Pentex (Kankakee, IL), fluorescein isothiocyanate, Goat anti-rabbit IgG, and glutaraldehyde were purchased from Sigma Chemical Co. (St. Louis, MO), and 3-aminopropyltriethoxysilane (APTES) from Pierce Chemical Co. (Rockford, IL).

2.2 Purification of Antibody.

Rabbit IgG was purified by gel filtration chromatography on a Sephacryl S-300 column (bed height: 94 cm, flow rate: 0.5 ml/min) equilibrated with 0.1 M phosphate-buffered saline (PBS, pH 6.8), and eluted with the same buffer. Eluant was monitored for absorbance at 280 nm and fractions containing the protein were pooled.

2.3 Preparation of Fibers.

Rabbit IgG was either passively adsorbed to non-silanized quartz fibers by incubating the fiber in 50 μ g/ml IgG in PBS (pH 7.4) for 30 min, 4° C or covalently linked to silanized fibers. Silanized quartz fibers were acid cleaned by immersion in a concentrated hydrochloric acid/methanol (1:1) solution for 30 min, followed by several rinses in distilled water. After washing, the fibers were soaked in concentrated sulfuric acid for 30 min and rinsed in distilled water. Fibers were then boiled 30 min in distilled water to remove any remaining acid and dried on a lint free cloth. Silanization of the fiber surface was performed by the method of Weetall.⁵ Cleaned fibers were incubated (75° C, 2 hr) in APTES (10% v/v, pH 3.5). Following incubation, fibers were washed with distilled water and dried overnight in a 115° C oven. Silanized fibers were then activated by incubating in glutaraldehyde (3%) for 90 min, followed by incubation in 50 μ g/ml solution of Rabbit IgG in PBS (pH 7.4) for 30 min, 4° C.

2.4 Fluorescence Measurements

A fiber optic evanescent wave sensor, designed and built by ORD, Inc. (North Salem, NH) was used for all experimentation. Quartz fibers, 1 mm in diameter with polished ends, were obtained from ORD, Inc. The sensor makes use of the evanescent wave effect by exciting a fluorophore just outside the waveguide boundary (excitation wavelength = 485/20 nm). A portion of the resultant fluorophore emission is trapped in the waveguide and is transmitted back up the fiber. This emitted wavelength is detected after transmission through 510 LP and 530/30 nm filters. The flow cell allowed the center 47 mm of a 60 mm long fiber to be immersed in 46 μ l which was exchanged every 14 sec (Figure 1).

After immobilizing the IgG, the fiber was placed in the flow cell of the instrument and perfused for 5 min with PBS containing bovine serum albumin (BSA) (0.1 mg/ml) to eliminate nonspecific binding. The fiber was then perfused with FITC-aR in PBS/BSA. Between experiments, the flow cell was washed in 1% SDS for 2 min followed by PBS for 10 min. Initial rates were determined graphically from tracing of the millivolt response vs time.

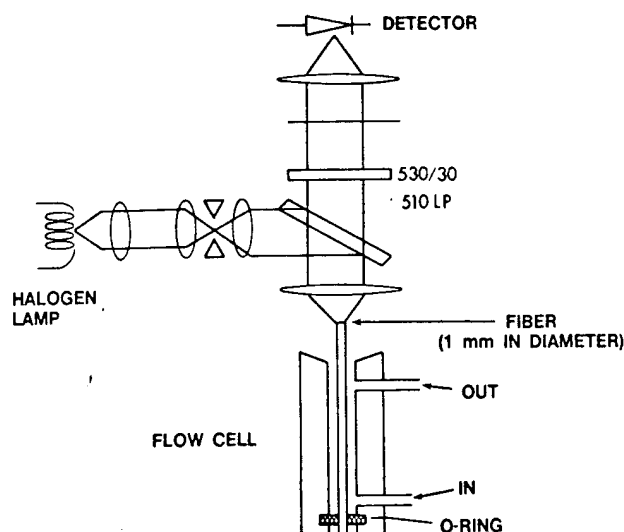


Figure 1. Schematic presentation of the Optical System used to Measure Fluorescence.

2.5 ¹²⁵I-Protein A Binding.

Rabbit IgG was immobilized on silanized fibers as previously described, pretreated with BSA, and incubated with ¹²⁵I-Protein A (specific activity- 0.3Ci/mmol, New England Nuclear) for 15 min. This represents the time required for fibers to reach equilibrium in the sensor. Silanized fibers without IgG were also incubated for determination of nonspecific binding. Fibers were washed in three passages of PBS and radioactivity was counted in an LKB Autogamma counter.

2.6 Fluorophore Labeling.

Toxin (1 mg) was reacted with 1 mg fluorescein isothiocyanate (FITC) on celite for 30 min at room temperature. After incubation, the conjugate was loaded onto a G-25 size exclusion column (25 x 1.1 cm) and eluted with PBS (pH 7.2). The labeled fractions were pooled and used in subsequent experiments.

3. RESULTS

Literature reports indicate that covalent immobilization of protein to fibers is preferred to passive adsorption due to the stable linkage that is formed and greater half-life of the sensor's responsiveness. Comparison between the covalent immobilization of IgG via the silanization procedure employed in this paper and passive adsorption of IgG onto bare quartz fibers revealed no significant difference in either initial or maximum binding when probed with FITC-Protein A (Table 1). Unless otherwise stated, all subsequent experiments were performed using the covalent immobilization of IgG on silanized fibers.

Table 1. Comparison of Silanized and Non-silanized Fibers

IgG incubation concentration	Silanized fibers		Non-silanized fibers	
	initial rate (mv/min)	total mv	initial rate (mv/min)	total mv
0.5 $\mu\text{g/ml}$	83	133	88	150
5 $\mu\text{g/ml}$	113	225	175	150
50 $\mu\text{g/ml}$	325	925	325	1150

FITC-labeled aR (0.5 $\mu\text{g/ml}$) bound nonspecifically to silanized quartz fibers as measured by evanescent fluorescence. However, with the addition of bovine serum albumin (BSA, 0.1 mg/ml) there was an 83% decrease of maximum binding. IgG coated fibers, pretreated with BSA and perfused with FITC-aR in PBS/BSA buffer yielded a maximum fluorescent asymptote over 200% of that obtained with the uncoated fiber (Figure 2).

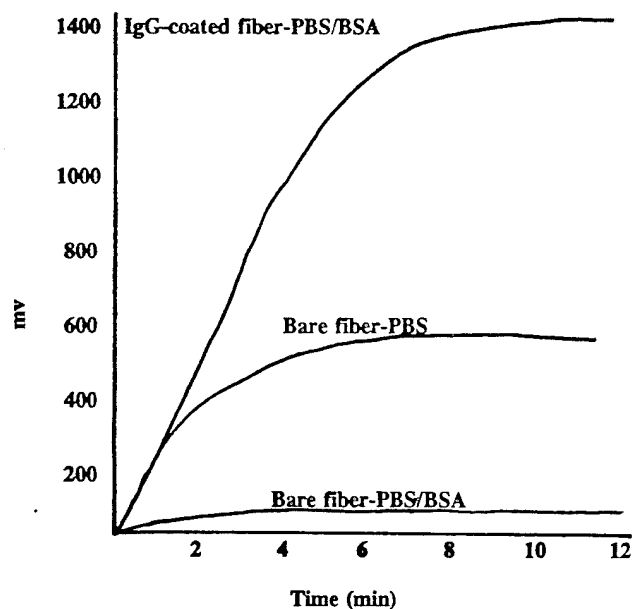


Figure 2. Specificity of FITC-aR for Rabbit IgG.

Perfusion of IgG coated fibers with increasing ratios of FITC-aR to unlabeled aR resulted in inhibition of the initial rate. With this competition-type assay, we were able to detect unlabeled aR at the nanomolar level (Figure 3). These data suggest that the binding of FITC-aR to IgG coated fibers is the result of specific binding of the aR to the IgG, rather than to the fiber itself.

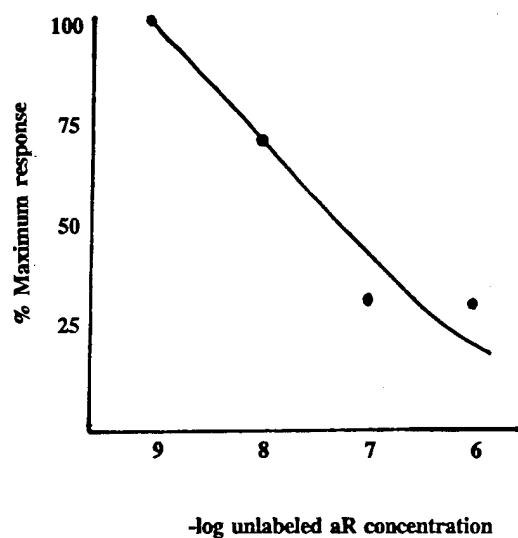


Figure 3. Inhibition of FITC-aR binding by unlabeled aR.

The IgG coated fibers bound FITC-Protein A in a concentration-dependent manner. Fibers incubated 30 min with 50 $\mu\text{g/ml}$ IgG bound 31 fmol ^{125}I -Protein A sites/fiber. Comparison of ^{125}I -Protein A and FITC-Protein A binding to fiber-immobilized IgG yielded similar slopes (Figure 4).

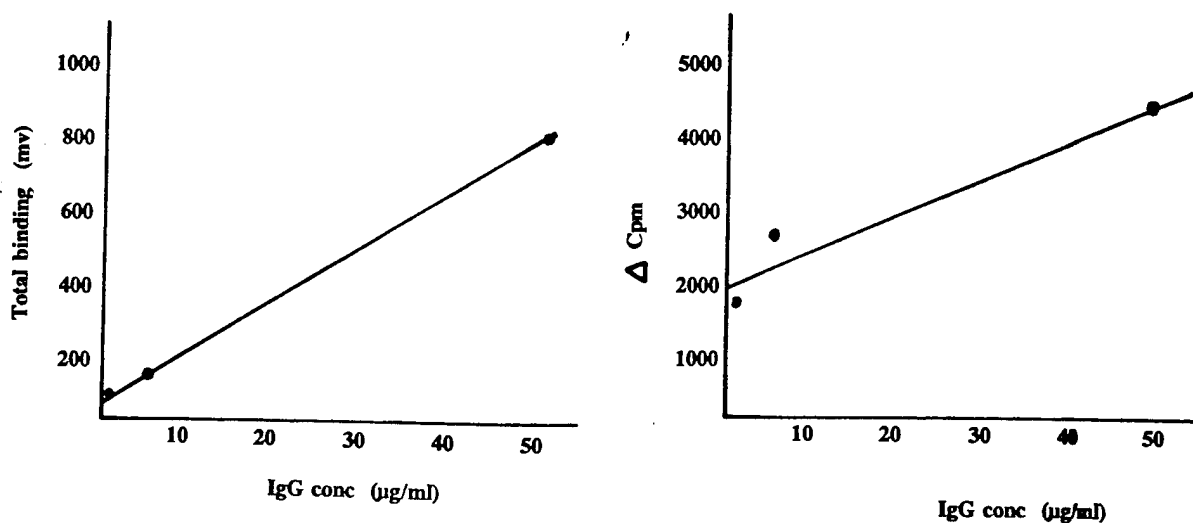


Figure 4. A- Binding of FITC-Protein A to IgG, B- Binding of ^{125}I -Protein A to IgG.

4. CONCLUSIONS

Fiber silanization produces a more stable immobilization by covalently linking the protein to the surface. The amount of biological material that binds to the silanized fiber appears to be dependent on several factors: the choice of silanization technique/crosslinker used, and the type of biomolecule used. The lack of difference we observed between non-silanized and silanized fibers may be due to the choice of an aqueous method of silanization and use of a glutaraldehyde activator. With aqueous silanization, lower amine loading occurs than when organic solvents are used, although the surface is reported to be more uniform. Preliminary experiments using organic solvent silanization with (3-mercaptopropyl)-trimethoxysilane (MTS) and N-(γ -Maleimidobutyryloxy)succinimide (GMBS) as a crosslinker yielded nearly twice the maximum binding compared with non-silanized fibers (unpublished data). Rogers reports the passive adsorption of 29 fmol/mm² of nicotinic acetylcholine receptor onto non-silanized fibers;⁶ however, covalent immobilization of nAChR yielded less.

Binding of fluorophores within the evanescent wave zone produces a fluorescent signal that is detected by the evanescent wave sensor. FITC-aR alone binds to the quartz fibers but this binding is almost entirely eliminated in the presence of 0.1 mg/ml BSA. In the presence of BSA, binding of FITC-aR on IgG coated fibers was 200% greater than on uncoated fibers. Cotreatment of fibers with labeled and unlabeled aR showed competition-like inhibition of FITC-aR. It may, therefore, be concluded that the observed fluorescence in the BSA-treated fibers is due to specific binding of FITC-aR to the IgG.

When probed with ¹²⁵I-Protein A, the silanized fibers bound 0.6 fmol/mm² Rabbit IgG. This value, compared with Bhatia's report⁷ of 6 fmol/mm² for immobilized IgG, may be due to a shorter fiber incubation time. The fiber optic evanescent wave sensor has the advantages of ease of use and reagent preparation. It is anticipated that new assays may be developed very quickly, since purification of antibodies and FITC-labeling of antigen are the only steps required for each new antigen. Fiber optics are not subject to interference problems, have a rapid response time, and are very versatile for developing new assays.

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